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Development and Characterization of Microsatellite and RFLP-Derived PCR Markers in Oat

Narinder Pal, Jagdeep S. Sandhu, Leslie L. Domier,* and Frederic L. Kolb

ABSTRACT

Two sources were evaluated for the production of polymerase chain reaction (PCR) markers for oat (*Avena* spp.). First, nucleotide sequences were determined for 250 unique clones from oat microsatellite-enriched genomic libraries. Forty-four of the 63 primer pairs designed were functional, of which 18 (41%) were polymorphic among 13 *Avena* species and six (14%) were polymorphic between oat cultivars Kanota and Ogle. Second, primers were designed from the sequences of six cDNA fragment length polymorphism (RFLP) probes. Primer pairs from all six cDNA clones were polymorphic among the 13 *Avena* species, three were polymorphic between Kanota and Ogle, but only one was polymorphic directly between 'Clintland 64' and IL86-5698. However, by cloning and sequencing the PCR products from Clintland 64 and IL86-5698, it was possible to identify nucleotide sequence differences at restriction enzyme cutting sites in DNA fragments from two other primer pairs. Using the two types of markers, we placed nine loci on the hexaploid oat restriction RFLP map. The RFLP-derived markers often mapped to the same or similar positions as the corresponding RFLP markers within and between mapping populations. The sequence analysis also revealed single nucleotide polymorphisms (SNPs) not at restriction enzyme cutting sites. Since multiple SNPs could be detected even within genes and the techniques for the development of SNPs and microsatellites are similar, it may be possible to identify more informative SNP markers than microsatellites from the same type of analysis.

Cultivated oat (*Avena sativa* L. and *Avena byzantina* C. Koch) is an important cereal crop with a large genome (1C DNA content $\approx 1.4 \times 10^{10}$ base pairs (bp); Bennett and Smith, 1976). It is a self-pollinated allohexaploid with a basic chromosome number of $n = 3x = 21$ that consists of three basic genomes (A, C, and D) (Rajhathy and Thomas, 1974). Breeders and geneticists are increasingly using molecular markers in oat and other plant species to study genome structure and genetic relationships and to manipulate genes. Microsatellites (Litt and Luty, 1989), or simple sequence repeats (Tautz et al., 1986), have emerged as important sources of ubiquitous genetic markers for many eukaryotic genomes (Wang et al., 1994). Microsatellites are tandemly repeated sets of 2 to 8 bp that can vary extensively in the number of repeats, which is believed to be due to slippage of DNA polymerase during replication, and/or unequal crossing over (Schlotterer and Tautz, 1992). The analysis of microsatellites is based on PCR, which is amenable to automation and much easier to perform than RFLP analysis. The reproducibility of microsatellites is such that they can be used efficiently by different research laboratories (via published primer sequences) to produce consistent data (Saghai Maroof et al., 1994).

Microsatellites are highly informative, locus-specific markers that have been reported from many plant species including maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), lettuce (*Lactuca sativa* L.) rice (*Oryza sativa* L.), sunflower (*Helianthus annuus* L), soybean [*Glycine max* (L.) Merr.], tomato (*Lycopersicon esculentum* Mill.), and wheat (*Triticum aestivum* L. em. Thell) (Senior and Heun, 1993; van de Wiel et al., 1999; Saghai Maroof et al., 1994; Wu and Tanksley, 1993; Brunel, 1994; Akkaya et al., 1995; Vosman and Arens, 1997; Roder et al., 1995, 1998). Being multiallelic and hypervariable, microsatellites have proven to be highly informative in studies of genetic and evolutionary relationships (Buchanan et al., 1994; Schlotterer and Tautz, 1992).

With the increasing availability of nucleotide sequence information from agronomic species, sequence tagged sites (STSs; Olson et al., 1989) and SNPs have become important sources of allele-specific markers for genome mapping, marker-assisted selection, and gene identification (Mano et al., 1999; Sanchez et al., 2000; Cho et al., 1999). In the absence of complete genome sequences, information from expressed sequence tags (ESTs) and end sequences from the inserts of large insert genomic clones are important sources of sequence information. Because of the similarities in genome organization and nucleotide sequences of genes among grass species (Devos and Gale, 2000), information gained in the analysis of rice, wheat, and barley should be applicable to the study and manipulation of the genomes of less well-studied species like oat.

Recently, microsatellite markers have been produced for cultivated oat (Li et al., 2000a,b). The objectives of this study were to (i) isolate and characterize additional microsatellite markers in oat, (ii) produce STS, and

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Abbreviations: bp, base pairs; AFLP, amplified fragment length polymorphism; BYDV, barley yellow dwarf virus; CAPS, cleaved amplified polymorphic site; ESTs, expressed sequence tags; LOD, likelihood of the odds; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; STS, sequence tagged site; Tm, melting temperature.

cleaved amplified polymorphic sequence (CAPS; Konieczny and Ausubel, 1993) markers derived from the sequences of cDNA RFLP probes, and (iii) map the markers on a molecular linkage map of hexaploid oat (O'Donoughue et al., 1995).

MATERIALS AND METHODS

Plant Materials

Thirteen oat cultivars (Clintland 64, 'Coast Black', 'Don', 'Golden', 'Gopher', 'Hazel', IL86-5698, 'IL86-6404', Kanota, 'Newdak', Ogle, 'Pennuda', and 'Terra') and 13 *Avena* species [*A. abyssinica* Hochst. (PI 58728), *A. barbata* Pott. ex Link. (PI 337986), *A. brevis* Roth. (CIav 9013), *Avena byzantina* (Kanota), *Avena eriantha* Dur. (CIav 9051), *A. fatua* L. (PI 544489), *A. longiglumis* Dur. (CIav 9071), *A. maroccana* Gdgr. (CIav 8330, 'Magna'), *A. nuda* L. (CIav 9009), *A. sativa* (Ogle), *A. strigosa* Schreb. (CIav 9112), *A. vaviloviana* Mordv. (PI 412733), and *A. wiestii* Steudel. (CIav 9053)] were used to investigate the levels of polymorphism detected by the PCR markers. Genomic DNAs were extracted from the above oat lines and 71 recombinant inbred lines of the Kanota × Ogle mapping population (O'Donoughue et al., 1995) as described by Doyle et al. (1990).

Library Construction and Microsatellite Retrieval

Microsatellite-enriched libraries were constructed as described by Prochazka (1996) with genomic DNA from the barley yellow dwarf virus (BYDV)-sensitive oat cv. Clintland 64 and 5′-biotinylated $(GAA)_8$, $(TCC)_8$, $(TGG)_8$, $(AG)_{12}$, $(AC)_{12}$, and $(AT)_{12}$ repeat probes. The PCR products were cloned directly into the pGEM-T vector with the TA cloning kit (Promega, Madison, WI). Plasmid DNAs were extracted by means of a Plasmid Mini Kit (Qiagen Inc., Santa Clarita, CA). In addition, 30 clones containing GAA repeats were provided by G. Scoles (University of Saskatchewan). Sequencing was performed by the dideoxy chain termination sequencing method (Sanger et al., 1977) and Applied Biosystems model 377 automated sequencers (PE Biosystems, Foster City, CA).

Primer Design and Evaluation

Oligonucleotide primers flanking the cloned microsatellites sequences were designed by means of PRIMER3 (Rozen and Skaletsky, 1997) and synthesized by GIBCO BRL (Gaithersburg, MD). A second set of primers were designed from the sequences of six cDNA RFLP probes (BCD1407, BCD1882, BCD1950, CDO270, CDO669, and CDO1158; Anderson et al., 2000) that are linked to loci for BYDV tolerance (Jin et al., 1998). The cDNA sequences were compared with plant genomic sequences in GenBank to identify conserved sequences and intron–exon borders. When similar genomic sequences were found, primers were designed within the conserved regions that flanked introns with the hope that they would identify sequences with length variation among oat species and cultivars. For the RFLP-derived primers that did not produce polymorphic bands in the Clintland 64 × IL86-5698 population, the PCR products were cloned and sequenced from the Clintland 64 and IL85-5698 parental lines as described above. Then the sequences from the two oat lines were compared with identify sequence differences that could be detected by restriction enzyme digestion.

PCR amplifications were performed in 20-μL reaction mixes containing 30 ng of oat genomic DNA; 50 m*M* KCl; 10 m*M* Tris-HCl (pH 9.0); 0.1% (v/v) Triton X-100; 0.2 m*M* each of dATP, dCTP, dGTP, and dTTP; 2.5 m*M* $MgCl_2$; 0.1 unit *Taq* DNA polymerase (Promega, Madison, WI); 1.5 pmol ^{33}P-labeled forward primer; and 1.5 pmol of unlabelled reverse primer. PCR was performed by means of one of the following two PCR conditions: (i) one cycle of 1 min at 94°C; followed by 25 to 30 cycles of 1 min at 94°C; 1 min at the Tm listed for each primer in Table 1; 1 min at 72°C, with a final elongation step of 72°C for 5 min; or (ii) a 'touchdown' PCR program consisting of 14 cycles of 1 min at 94°C; 1 min at 65°C; and 1 min at 72°C. Annealing temperatures were decreased 0.7°C each cycle to 55°C. PCR continued for 30 additional cycles of 1 min at 94°C; 1 min at 55°C; and 1 min at 72°C with a final elongation step of 72°C for 5 min. PCR products were separated on 6% (w/v) denaturing polyacrylamide gels (FMC Bioproducts, Rockland, ME) at a constant voltage of 1200 V for 3 to 4 h. Gels were dried and exposed to x-ray films (BIOMAX; Eastman Kodak Co., Rochester, NY) at room temperature for 1 to 2 d.

Linkage Analysis

Linkage analysis and map construction were performed by MAPMAKER version 3.0 (Lander et al., 1987) and a Kanota × Ogle data set (Siripoonwiwat et al., 1996). Markers were grouped with a log-likelihood of the odds (LOD) score of 8.0 and maximum recombination level of 0.30. Three-point analysis was used to order loci within groups. Once a map order had been established, linked markers were assigned to intervals at a LOD score of 2.0. Map distances were calculated as previously described (Jin et al., 1998). Mapped oat microsatellite loci were numbered and designated AM for '*Avena* microsatellite' as described by Li et al. (2000a,b).

RESULTS

Characterization of Microsatellite Clones

Of the 306 cloned inserts sequenced from di- and trinucleotide repeat microsatellite-enriched libraries, 250 (82%) were unique and contained microsatellite repeats. One hundred fifty-five (62%) clones had perfect repeats. Three types of dinucleotide, $(AC)_n$, $(AG)_n$, and $(AT)_n$, and two types of trinucleotide, $(CAA)_n$ and $(GAA)_n$, repeats were analyzed in this study. $(AC)_n$ repeats were the most prevalent followed by $(AG)_n$ and $(AT)_n$. A single $(CAA)_n$-containing clone was isolated. All $(GAA)_n$-containing clones were provided by G. Scoles (University of Saskatchewan). Repeat length ranged from 2 to 69 for dinucleotide repeats and from 3 to 60 for trinucleotide repeats. In about 30% of the clones, sequences flanking the repeats were not of sufficient length or complexity to design specific primers. Primer pairs were designed from 63 unique microsatellite flanking sequences.

Out of the 63 pairs of primers, 44 (70%) produced amplification products of the expected sizes at annealing temperatures ranging from 55 to 60°C (Table 1). The sizes of the products varied from 102 to 341 bp with an average size of 198 bp. The 44 primer pairs were used to detect polymorphisms among 13 *Avena* species and 13 oat cultivars. Similar to the results of Li et al. (2000a), none of the nine primer pairs designed to clones containing trinucleotide repeats detected polymorphisms among the oat species evaluated. Eighteen primer pairs

Table 1. Oat microsatellite and RFLP probe-derived primers.

Name	Sequence	Repeat	Expected size	Tm	Polymorphic†	Linkage group(s)‡
			bp			
AM81	ACGGGCTACCCCTGTTAGAA GGGACACATGCAATCACCTA	$(AC)_9$	174	58	No	–§
AM82	CACAAGTTGGGGCACTATCC GGAGGAAGACCACGAGAACA	$(AC)_9$	220	58	No	–
AM83	CACTGCCATACATTCTGTCG CCTCTACCGCAAAGGAAGAA	$(AC)_{11}$	190	55	Yes	30
AM84	GATAACAACTTGGATGCAACTGA CAGGTTGACAAGGGAACGAT	$(AC)_9$	163	55	Yes	–
AM85	CACCAAGACGATTCGCTACA CTTTTGCAAGACCTTCAAATCA	$(AC)_{10}$	186	55	No	–
AM86	ACAACTCCTTGCGGGTCATA AGTGCATGCGAATTTTTCAG	$(AC)_9$	194	55	No	–
AM87	GAGCAAGCTCTGGATGGAAA CCCGTTTATGTGGTTGTTAGC	$(AC)_{13}$	150	55	Yes	24
AM88	ACATCGCCGAACATATTGCT CATATCCTGACCGGGTCCT	$(AC)_8$	162	58	No	–
AM89	GGCGGTTGGAGAGTGTCTT AGGTGAAGGCGAGTGGAAG	$(AC)_{16}$	193	58	No	–
AM90	GTCGGATGCGTATCTCTCGT GAGAAGCTGCCGGTACCATA	$(AC)_{15}$	191	55	Yes	–
AM91	GCGATTTACAACGACCTTATGA CCAGTGCTTCTGATCTGGAAT	$(AC)_{20}$	171	55	Yes	37
AM92	CCGCACTATCGGCTTGTATC GTGGGAATCGAGGGTAGAGC	$(AC)_{13}$	130	60	Yes	–
AM93	CCGACATTGCATATGCTGTTA TTGATAACCAAAGGCAGATTATGA	$(AC)_{12}$	186	55	No	–
AM94	TCTGGAAGTGGTAGTGGGTGT AACCCATCCACCATAGGC	$(AC)_{11}$	187	58	No	–
AM95	GGCAGGAAGTGGGTCAGATA CTGAGAACTCGGCCTAGGAA	$(AC)_{13}$	233	55	Yes	–
AM96	CCAAAAGGCCGATTGTGATA AACCCTCTTTTGCGGTCAAT	$(AC)_{10}$	215	55	No	–
AM97	TGGGTAGTCCTGGAGCTGAAGCG GCCATACGACGAGCACCACAAAG	$(AC)_{35}$	341	60	Yes	–
AM98	GATCTTGGCGTCGGGCGTTAG AGTAACAACCGAGTGCGATGCTCC	$(AC)_{10}$	229	55	Yes	–
AM99	GCAATCATTCCAACTTGTTATGACACA GATAGTGGAAGCGGCATCAACATC	$(AC)_{12}$	169	58	Yes	–
AM100	CGCTGTCCAATGTTCCACTCCTG CAGTTGATAACCAAAAGGCTGATTGTG	$(AC)_{15}$	181	58	No	–
AM101	CGCTTATCACCCACAGACCGAC CACAATCTGCCTTTGGTTATCAACTG	$(AC)_3$	209	58	Yes	–
AM102	TGGTCAGCAAGCATCACAAT TGTGCATGCATCTGTGCTTA	$(AC)_9$	213	55	Yes	22
AM103	CAACAACTTGGATGCAACAGA CATCAACGTGAGGTTCTCCA	$(AC)_{16}$	212	58	Yes	–
AM104	AACAATGATGGGGATGGTGT GTCGTGAGCAAGTTGAACCA	$(AG)_{36}$	186	55	Yes	–
AM105	GTCGCGGGTAGCTTAATGG ACTGTGATCCAAGTCGTCCA	$(AC)_2(AC)_5(AC)_2$	192	58	No	–
AM106	CAAGCATCTGCAGACTCACC GGGAACCAGTTAGGCTCAAA	$(AC)_4(AC)_5$	166	58	No	–
AM107	CCGTGGTATGAGTGCTAATGAA TTTTCATGAGGTTGTCCTTGG	$(AC)_3(AC)_4$	263	58	Yes	–
AM108	ATCCCAAGTACACCCCTTCC GCACAATGGCATGACGTAAC	$(AG)_5(AG)_2$	192	55	No	–
AM109	ACCCCTGTCAGAGGAACGTC GGGACACATGCAAGAACCTA	$(AC)_8(AG)_5$	178	58	No	–
AM110	TTCATCGCTGTGACCATGTG TCCGTGAGTTCCCTCAGTTC	$(AC)_3(AG)_4$	193	58	No	–
AM111	CAAGTGGGCGGAGTTAGGTA TCTTCGGTGTGAGTTTCCAA	$(AC)_{11}(AT)_6$	235	58	No	–
AM112	AGCGGTGTAGGGGAAAGAGT TTCTTGGTTTAGATGGGAGGA	$(AG)_3(AC)_9(AT)_8$	234	55	Yes	2
AM113	ATCAAAGATCGCCTCGAGTT GGTCCAACATAGGCACAAGG	$(AG)_{15}(AC)_9$	228	55	No	–
AM114	TTCGACAGTGAGTTATCCTTGC CAATGCATGGCACAGTCAA	$(AG)_{24}(AC)_{14}$	206	55	Yes	–
AM115	CGCAACTCTTCCTACTTTTTGTT TGGCAAACTCCCTCGATTTA	$(AC)_9$	214	55	Yes	23
AM116	TCGAAAATCAGAAGTTATACAACAAAA TCAATCTCAAGAACCTGGTAAGCA	$(CAA)_{58}$	238	60	Yes	–
AM117	CACTACCCGCAAAGTCATCA AGGACACATTTCCAGCAAGG	$(GAA)_{14}$	237	58	No	–

Continued on next page.

Table 1. Continued.

Name	Sequence	Repeat	Expected size	Tm	Polymorphic†	Linkage group(s)‡
			bp			
AM118	TCCATTGCATGACCTCTTGA ACTTCCCGCAAAATCATCAC	$(GAA)_8$	190	58	No	–
AM119	GCATTGGCTTTGTCCCTAAA GTTTTGCCATTCGTGGACTT	$(GAA)_{11}$	118	58	No	–
AM120	GCAAGCAAAGGACGAACAAT TGGGAACCCAAATAGACCAA	$(GAA)_{12}$	268	58	No	–
AM121	GGAATTGGCTTTGTCTCCAA TGGTCTTACTCGCCACAATG	$(GAA)_{16}$	121	58	No	–
AM122	TAGCTAAGGCCACCTTTCCA TTAGCACTTCCCGCAAAATC	$(GAA)_6$	218	58	No	–
AM123	TAATTTCCTCCCTGACCGTG CTCAGACCTGCGAACAAACA	$(GAA)_6$	253	58	No	–
AM124	TTTGAAGGATCAACTTGCCA AGGGTTAGGCCATTTGGAAC	$(GAA)_{19}$	123	58	No	–
270-1F	GCAATTTAATCAAGCATCCAAAATTCATG		>800	55	Yes	2 (1445), 3 (774)
270-1R	CGAGAAACAGCAAGAACACCACCAAC					
270S-1F	ATTCTTGTTGGTGGTCGCTTGGTG	$(AC)_7$	183	55	ND¶	ND
270S-1R	AACTGCTACACCCACATACCCACAGG					
669-1F	GCTTTCCCTTTGCTAGAGCTTGGA		>100	55	Yes	–
669-1R	TCCTGCTGGAAAACATGGCACA					
1407-1F	GCAGCATATTTGGAAACAACTTAAGAAACAC		290	55	Yes	–
1407-1R	CTTCGAGCTGTTGCTTCCAATGAG					
1158-1F	GCTCTTGGTACAGCAAAAGCCTTAGAGTA		>380	55	Yes	–
1158-1R	TCTCCACGGAATAGAAGGATCAACCA					
1882-1F	CAACACCAGTTCAAGATTACCCTATGAAGA		>800	55	Yes	2 (940), 36 (885)
1882-1R	TGCTCCTGATCTTAAGGTCCATAAAGTCG					
1950-2F	CCACCTACGTCGTCGGCGTCAATG		>380	55	Yes	–
1950-2R	GTCGCGGAACGCCTGGTTCACCTC					

† Markers were polymorphic among the 13 *Avena* species.
‡ Kanota × Ogle linkage group to which markers mapped.
§ Markers were not polymorphic between Kanota and Ogle.
¶ ND = not determined.

designed from clones containing dinucleotide repeats produced bands that were polymorphic among the 13 *Avena* species (Table 1). Six (14%) of these primer pairs (AM83, AM87, AM91, AM102, AM112, AM115) produced bands of the expected sizes that were polymorphic between Kanota and Ogle (Fig. 1A) and many of the other oat cultivars. Five additional primers produced major monomorphic bands with fainter polymorphic bands above or below the major bands. However, the faint polymorphic bands were not always observed and were not analyzed further.

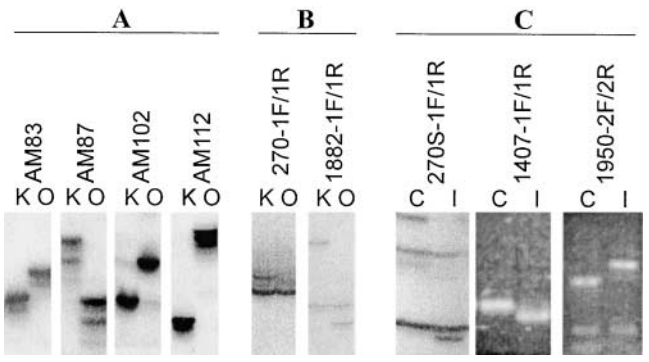

Fig. 1. Examples of polymorphisms observed between oat lines with microsatellite and RFLP-derived PCR markers. (A) Microsatellite markers 83, 87, AM102, and AM112. (B) STS markers derived from CDO270 (one of two loci ± polymorphic) and BCD1882 (two of three loci ± polymorphic). (C) Microsatellite marker derived from CDO270 and CAPS markers derived from BCD1407 and BCD1950 cleaved with *Mse*I and *Mbo*I, respectively. The names of the primers are listed above each image. The oat lines analyzed are indicated above each lane K = Kanota, O = Ogle, C = Clintland 64, and I = IL86-5698.

Primer pairs derived from all six of the RFLP probes yielded multiple polymorphic bands among the 13 *Avena* species. Between Kanota and Ogle, three (p1882, p270, and p1158) showed presence-or-absence polymorphisms between (Fig. 1B). Only one of the RFLP derived primer pairs, 270-1F/1R, was directly polymorphic between Clintland 64 and IL86-5698. Sequence analysis of the products synthesized by primers 270-1F/1R showed that one of the bands contained a short $(AC)_7$ repeat. Because the PCR fragment produced by 270-1F/1R was over 1 kb, a second set of primers (270S-1F/1R) were designed to flank the repeat and produce a 183-bp fragment. The two new primers detected a codominant polymorphism between Clintland 64 and IL86-5698 in one of the three bands amplified (Fig. 1C). The CDO270 sequence was the only RFLP-derived sequence that contained an obvious microsatellite repeat. Even so, nucleotide sequence analysis of the fragments amplified by 1407-1F/1R and 1950-2F/2R from Clintland 64 and IL86-5698 identified single nucleotide polymorphisms (SNPs) between the two sequences, some of which occurred in restriction enzyme recognition sites. Using this information, CAPS markers were produced by digesting the PCR products from primers 1407-1F/1R and 1950-2F/2R with *Mbo*I and *Mse*I, respectively (Fig. 1C).

Linkage Analysis

The segregation of banding patterns derived from the six microsatellite primer pairs that produced single polymorphic bands and three RFLP-derived primer pairs that showed polymorphism between Kanota and

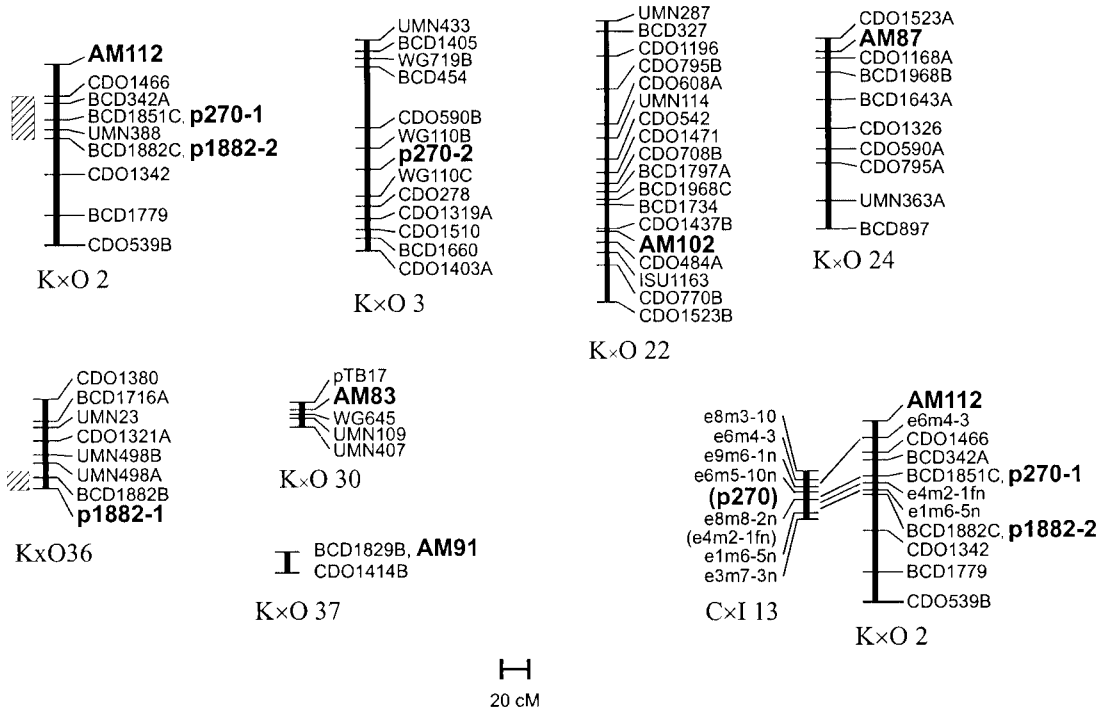

Fig. 2. Linkage map of PCR markers in the Kanota × Ogle recombinant inbred line population. Map distances are given in centimorgan (Kosambi function). Markers added in this study are shown in bold. Markers in parentheses have been assigned to intervals only. The positions of quantitative trait loci for tolerance to barley yellow dwarf virus are indicated with hatched boxes. In the lower right portion of the figure, the relative map positions of PCR markers derived from CDO270 in the Kanota × Ogle (K×O) and Clintland 64 × IL86-5698 (C×I) populations are shown.

Ogle were evaluated in the Kanota × Ogle mapping population. The nine polymorphic bands scored were assigned to one of seven linkage groups and mapped to unique positions (Fig. 2).

Most of the markers mapped at LOD score values greater than 6.0. Marker AM91 mapped at the same position as a RFLP marker on linkage group 37. RFLP-derived markers p1882-1, p1882-2, p270-1 and p270-2 mapped either close to (distance less than 5 centimorgans) or at the same position as the corresponding RFLP markers. In the Clintland 64 × IL86-5698 population (Jin et al., 2000), p270 mapped to a position corresponding to p270-1 in the Kanota × Ogle population (Fig. 2). Marker p1407 was linked to markers in Clintland 64 × IL86-5698 linkage group 16, for which a corresponding linkage group in the Kanota × Ogle population has not been identified yet. Finally, p1950 was unlinked to previously mapped markers.

DISCUSSION

A set of 250 microsatellite-containing clones was isolated from enriched libraries constructed from Clintland 64 genomic DNA. A high percentage of clones (62%) contained $(AG)_n$, $(AC)_n$, or $(AT)_n$ dinucleotide repeats, which is similar to earlier reports of microsatellite clone recovery in wheat (Bryan et al., 1997) and rice (Panaud et al., 1995). As previously reported for barley, wheat, and rice, $(AC)_n$ repeats were more common than $(AG)_n$ in our study in oat (Liu et al., 1996; Ma et al., 1996; Panaud et al., 1995). $(AT)_n$ repeats usually represent the most common type of repeat in plants (Powell et al., 1996; Wang et al., 1994), but were isolated only rarely in this study. This may be because $(AT)_n$ repeats are palindromic and therefore may not have been efficiently enriched during the capture process. Indeed, both clones that contained $(AT)_n$ repeats also contained $(AC)_n$ repeats, which may have facilitated their capture.

Primer pairs were designed from a subset of the unique microsatellite clone sequences. Most (65%) of the primer pairs produced bands of the expected sizes. Similar success rates were reported for sorghum [*Sorghum bicolor* (L) Moench; Brown et al., 1996] and wheat (Roder et al., 1998). Six of the primer pairs produced polymorphic markers that were placed on the Kanota × Ogle map. We interpreted the faint polymorphic bands sometimes associated with strong monomorphic bands as unique sequences and the strong monomorphic bands as repeated sequences. These predictions are supported by the observations of Li et al. (2000a) who found that 42% of their randomly selected *Avena* microsatellite clones contained repeated sequences. Comparison of the clones analyzed in this study to ones in GenBank showed that three clones contained retrotransposon-like sequences and another five clones were similar to dispersed-repeated elements (data not shown). None of the clones containing repeated sequences were polymorphic between Kanota and Ogle. Interestingly, AM83 was polymorphic, but nine clones nearly identical to it were sequenced. Of the functional primer pairs, 41% (18 out of 44) detected polymorphisms among 13 *Avena* species. Only 14% of the functional primer pairs detected intraspecific poly-

morphisms, which is similar to frequencies detected by AFLPs (Jin et al., 2000), but less than that of RFLPs on the same material (O'Donoughue et al., 1995). The low level of polymorphism observed with the microsatellites could be because of the types of repeats analyzed or, as suggested by Li et al. (2000a), the repetitiveness of the cloned sequences.

No clear relationship between total repeat length and degree of polymorphism was observed in this study. Very long trinucleotide repeats, like $(CAA)_{58}$ in AM116, were monomorphic, whereas shorter dinucleotide repeats, like $(AC)_9$ in AM115, were polymorphic. This is in contrast to results for wheat (Bryan et al., 1997) where the degree of polymorphism increased with the total length of the repeat.

All six of the PCR markers derived from sequences of RFLP probes were polymorphic among the 13 *Avena* species compared with just 41% of the randomly selected microsatellite markers. This is a small sample size and the RFLP sequences analyzed had been "preselected" to be polymorphic by virtue of having been mapped, which meant that they had already been shown to be polymorphic between Kanota and Ogle. In addition, the RFLP probes were selected because they represented unique or low copy sequences—a factor which is likely more important in predicting the potential informativeness of markers. It was not determined how often primers from randomly selected cDNAs would be polymorphic, but the level of polymorphism probably would be similar to that of randomly selected RFLP probes.

The conversion of the RFLP markers to STS markers frequently preserved the informativeness of the original markers. In the case of BCD1882, both of the loci that were polymorphic as RFLP probes were polymorphic as STS markers. Similarly, the third band, presumably representing a third locus, was monomorphic with both techniques. In addition, the markers often mapped to the same or similar positions as the corresponding RFLP markers within and among mapping populations (e.g., p1882-1, p1882-2, p270-1, and p270-2). In these studies, we were able to use sequence information from the amplified bands to identify RFLPs within monomorphic PCR fragments. It also should be possible to use this type of sequence information to develop SNP markers, like molecular beacons (Tyagi and Kramer, 1996), for use in marker-assisted selection for BYDV tolerance loci.

With the determination of thousands of sequences from rice and Triticeae EST clones (Yamamoto and Sasaki, 1997; Anderson et al., 2000), the ability to produce gene-specific probes from published sequences is growing rapidly. Since multiple SNPs could be detected even within the coding regions of genes and the techniques for the development of SNPs and microsatellites are similar, it may be possible to identify more informative SNP markers than microsatellites from the same level of analysis. In this study, microsatellites were no more polymorphic than other markers. However, if the microsatellite libraries were prescreened for unique or low-copy sequences, the number of polymorphic markers likely would be increased.

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